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Nutritional and *In vitro* Bioactive compounds analysis of *Canthium coromandelicum* (Burm.f.) Alston leaves

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Abstract
Canthium coromandelicum (Burm. f.) Alston leaves are widely used in ayurvedic medicine. Present study was conducted to quantify the proximate, mineral content and selected secondary phytochemicals which were present in *Canthium coromandelicum* (Burm. f.) Alston leaves. Dried leaves of *Canthium coromandelicum* (Burm. f.) Alston was used to analyse proximate composition, AAS analysis and qualitative as well as DPPH Scavenging activity was carried out to quantify the constituents. According to the results of the study, leaves contained alkaloids, flavonoids, saponins and polyphenolic compounds while total polyphenol content was observed as 17.27±0.19 mg/GAE/100g along with total alkaloid content, total saponin content and antioxidant content (IC_{50} valve) were detected as 0.007±0.00mg/g, 0.10±0.01 mg/g and 10.88 mg/ml respectively. Percentage of moisture, carbohydrate, crude protein, crude fiber, ash content and crude fat content were accounted for 65.21±1.54%, 12.17%, 8.27±0.89%, 6.41±1.18%, 6.17±0.07% and 1.77±0.14% respectively. *Canthium coromandelicum* leaves contained 461.89mg/100g of Potassium, 348.47mg/100g of Calcium, 46.71mg/100g of Sodium and 10.19mg/100g of Fe. Based on the findings, *Canthium coromandelicum* (Burm. f.) Alston leaves have potential for fulfilling both dietary and medicinal requirement.

Keywords: *Canthium coromandelicum* (Burm.f.) Alston, alkaloids, polyphenol, saponin, flavonoids

1. Introduction
Canthium coromandelicum (Burm.f.) Alston leaves are consumed as a leafy vegetable both raw form "Sambal" and cooked form "Mallum" in Sri Lanka and which is recommend to consume for preventing ailment specially diabetes [1]. *Canihium coromandelicum* is small tree or much-branched shrub and belongs to family Rubiaceae [2]. "Kara" is common name for C. *coromandelicum* in Sinhala and "Karai" in Tamil. 230 of *Canthium spp.* [3] have found in different distributed areas including Sri Lanka, India and tropical East Africa [4]. Ethnomedicinal uses of this plant as whole have been reported as preventing diabetes [5], control high blood pressure and reduce unwanted fat [6], antioxidant and diuretic activity [7], use to treat to scabies and ring worm infection and snake bite [8]. D. Sasmal and coworkers [9] have reported that preclinical pharmacological activities as antimicrobial and anti-HIV activities, hypocholosterolaemic activity, oral hypoglycaemic activity.
The aim of study is to identify and quantify the nutritional/dietary values as well as selected medicinal properties of *Canthium coromandelicum* (Burm.f.) Alston leaves which grow in Sri Lanka.

2. Materials and methods
2.1 Collection and Authentication of *Canthium coromandelicum* (Burm.f.) Alston leaves
Canthium coromandelicum (Burm.f.) Alston leaves were collected and screened for fresh and healthy leaves and then washed with chlorinated water followed by distilled water. Then, herbarium specimen was prepared and authenticated by National Herbarium Peradeniya, Sri Lanka.

2.2 Preparation of methanolic extract
Washed leaves were dried for 7 days at room temperature in a shade room and then pulverized to obtain coarse powder. 20g of powdered sample was extracted with 250ml of 80% methanol/water followed by evaporation using rotary evaporator at 40 ^{0}C.

2.3 Preliminary phytochemical investigation [10, 11, 12]
Test for Alkaloids
Wagner's test: 5 drops of Wagner's reagent were added to 2 ml of extract
Mayer's test: Few drops of Mayer's reagent were added to 1 ml of extracted solution

Test for Flavonoids
Ferric chloride test: 2 ml of 10% $FeCl_3$ was added to 2 ml of extract

Test for Saponins
Frothing test: 100mg of powdered sample was added to 20ml of distilled water followed by shaking well for 30 min

Test for Tannin
Ferric chloride test: 5 drops of 10% $FeCl_3$ was added to 2 ml of extract
Gelatin test: 5ml of distilled water, 2 ml of 1% gelatin solution

and added to 50 mg of powdered sample with 2 ml of 10% of sodium chloride

Test for Phenolic compounds
Lead acetate test: 5 drops of 1% lead acetate was added to 1 ml of extract

Test for Carbohydrates
Fehling's test: 2 ml of distilled water was added to 2ml of extract and followed by addition of 2ml of Fehling's A & B and heated until it was boiled
Bradford's test: 4 drops of Bradford's reagent was added to 2 ml of extract

Test for Protein
Millan's test: 6 drops of Millan's reagent were added to 2ml of methanolic filtered extract

2.4 Quantitative analysis
Proximate analysis
Analysis of proximate composition was conducted by referring standard protocols (AOAC 2012) published by Standard procedures of Association of Official Analytical Chemist [13]. Moisture analysis was conducted using oven drying method at 105^{0}C for 3 hours, crude fat, crude fiber and crude protein and ash content were analyzed according to Soxhlet extraction with petroleum ether, digesting the defatted sample with 1.25% (w/v) of H_2SO_4 and 1.25% (w/v) of NaOH, micro Kjeldhal method and dry ashing method respectively described by AOAC, 2012.
Determination of Mineral content:
Mineral content was analyzed according to Atomic Absorbance Spectrophotometric (AAS) method and dry ashing method described by AOAC, 2012 [13]. Concentration of calcium, potassium, sodium and iron were determined by incineration of 3 g of dried leaves and placed in a muffle furnace at 550 ^{0}C until it converted into ash then addition of hot HNO_3 and 10% HCl (1:3) to dissolve ash and volume up to 100 ml in a volumetric flask using distilled water. The absorption values were read and mineral content were expressed as mg/100g.

2.5 Quantification of bioactive compounds
Total phenol content
ISO 14502-1 method [14] was referred to determine the total phenol content of *Canthium coromandelicum* (Burm.f.) Alston leaves. 1.0 ml of extract (70% methanol extract) was mixed with 5ml of 10% Folin-ciocalteu's phenol solution with 4 ml of 7.5% sodium carbonate solution followed by heating for 10 min at 40 ^{0}C and then absorbance was measured at 765 nm. Standard curve was constructed using Gallic acid and total phenol content was calculated as mg/GAE/100g.

Total saponin content
Total saponin content was analyzed according to protocol describe by Obdhoni and Ochuko [15] and expressed as (mg/g). 10g of dried powder was added to known amount of 20% ethanol followed by heating at 55 ^{0}C for 4 hours then re-extracted and concentrated to 40 ml and then addition of diethyl ether and shaken. Then 60 ml of n-butanol was added followed by adding 5% aqueous NaCl then evaporated and final weight of sample was measured.

Total alkaloid content
Gravimetric method described by J.B. Harbone [16] was used to determine the total alkaloid content and expressed as mg/g. 5 g of dried powder of sample was added to 100ml of 10% acetic acid/ethanol solution and allow to stand for 8 hours. Then, sample was filtered followed by concentration to one – fourth of volume at 80 ^{0}C. Conc. NH_4OH was added drop-wise until complete precipitation. Then, solution was filtered and precipitate was dried and weighed.

Antioxidant content
In vitro antioxidant activity was analyzed according to Blois method with some modification [17]. The methanol extract concentration series (2.5 – 40 mg/ml) was prepared and 2.5 ml of DPPH was added followed by covering with Al foil and kept for 30 min in a dark place. Gallic acid was used to construct the standard curve. The absorbance was measured at 517 nm using UV-Vis spectrophotometer. IC_{50} value was calculated as follows:

$$\% \text{ Inhibition} = (\text{Blank absorbance} - \text{Sample absorbance}) / (\text{blank absorbance}) \times 100$$

4. Results and Discussion
Preliminary phytochemical investigation results were tabulated in table 01 as follows;

Table 1: Preliminary phytochemical investigation

Phytochemical	Test/s	Methanol extract
Alkaloid	Mayer's test	+
	Wagner's test	+
Phenolic compounds	Lead acetate test	+
Flavonoid	Ferric chloride	+
Saponin	Frothing test	+
Tannin	Ferric chloride test	-
	Gelatin test	-
Protein	Millan's test	+
Carbohydrates	Fehling's test	+
	Bradford's test	+

(-) Absence; (+) Presence

All the screened phytochemicals without tannins were observed in previous study [3]. These compounds have been reported for various medicinal and nutritional values including; alkaloids have antifungal properties, antibacterial and anti-parasitic activity [18, 19]. 0.007±0.00mg/g of alkaloids were present in *Canthium coromandelicum* (Burm.f.) Alston leaves while saponin content has obtained as 0.10±0.01 mg/g. Anticancer activity, anti-cardiovascular activity and anti-inflammatory activities [20] which are medicinal activities showed in saponins. Flavonoids and polyphenols act as natural antioxidants and they have different biological and medicinal activities such as anticancer [21]. Anti-allergic, anti-diabetes [18], antiatherosclerotic activities [22, 23, 24] were reported in flavonoids. Calculated antioxidant content (IC_{50} value) of *C. coromandelicum* was 10.88 mg/ml and total polyphenol value was 17.27±0.19 (mg/GAE/100g).

Table 2: Obtained values for quantification of phytochemical

Phytochemical	Quantity
Total polyphenols	17.27±0.19 mg/GAE/100g
Total alkaloids	0.007±0.00mg/g
Total saponins	0.10±0.01 mg/g
Antioxidant content (IC_{50} value)	10.88 mg/ml

Table 3: Proximate analysis results of *C. coromandelicum* leaves

Constitute	Quantity (%)
Moisture	65.21±1.54
Crude fat	1.77±0.14
Crude fiber	6.41±1.18
Crude protein	8.27±0.89
Ash	6.17±0.07
Carbohydrates	12.17

Table 03 indicates the results of proximate analysis of the sample. According to the results, the highest percentage was observed for moisture as 65.21±1.54%. Carbohydrates which act as energy source as well as structural components in cell [25] was observed as 12.17%. Third highest percentage was observed for crude protein as 8.27±0.89% which also has similar function as carbohydrates and it plays a vital role for bone health, renal health, provide essential amino acids [26]. The obtained results for crude fiber and ash content were almost similar which were 6.41±1.18% and 6.17±0.07% respectively. Crude fiber is essential for maintaining bowel health and it reduces the risk of heart disease, type 2 diabetes as well as prevent colorectal cancer [25]. This plant contained minute amount of crude fat which was observed as 1.77±0.14%. Fat is beneficial for storing fat soluble vitamins including vitamin A, D, E, K analysis of ash content is the initial step for determine the individual ion concentration which present in the sample [27].

Mineral content (Na, Ca, Fe and K) of *Canthium coromandelicum* (Burm. f.) Alston leaves was tabulated in table 04 as given below:

Table 4: Mineral composition of *Canthium coromandelicum* (Burm.f.) Alston leaves.

Type of ion	Quantity of ion (mg/100g)
K	461.89
Ca	348.47
Na	46.71
Fe	10.19

The highest content of mineral was observed as K (461.89mg/100g) which is a blood mineral and necessary for maintain fluid balance as well as secretion of some hormones and activate of enzyme [28]. Calcium was shown the second highest concentration among these four metal ions. Ca needs to grow and maintain of teeth and bone as well as necessary for blood coagulation [29]. Na ion content of the sample was recorded as 46.71 mg/100g while Fe was 10.19 mg/100g. Fe plays an important role for producing hemoglobin and iron deficiency causes anemia [30].

5. Conclusion

Based on the findings, *Canthium coromandelicum* (Burm.f.) Alston leaves contained beneficial phytochemicals such as alkaloids, flavonoids, polyphenols and saponins as well as dietary components including proteins, carbohydrates, fiber as well as minerals. Overall, it can be concluded that *Canthium coromandelicum* (Burm.f.) Alston leaves can be used to fulfil both nutritional and medicinal requirements.

6. References

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